Theor Appl Genet (2004) 108:525–534
DOI 10.1007/s00122-003-1451-5

ORIGINAL PAPER

G. Lu · C. Jantasuriyarat · B. Zhou · G.-L. Wang

# Isolation and characterization of novel defense response genes involved in compatible and incompatible interactions between rice and *Magnaporthe grisea*

Received: 25 March 2003 / Accepted: 20 August 2003 / Published online: 5 November 2003
© Springer-Verlag 2003

**Abstract** To identify early-induced defense genes involved in broad-spectrum resistance to rice blast, suppression subtractive hybridization was used to generate two cDNA libraries enriched for transcripts differentially expressed in *Pi9(t)*-resistant and -susceptible plants. After differential screening by membrane-based hybridization and subsequent confirmation by reverse Northern blot analysis, selected clones were sequenced and analyzed. Forty-seven unique cDNA clones were found and assigned to eight different groups according to the putative function of their homologous genes in the database. These genes may be involved in pathogen or stress response, signal transduction, transcription, cell transport, metabolism, energy or protein destination. Northern blot analysis showed that most of these genes were induced or suppressed after blast infection, and that half of them showed differential expression patterns between compatible and incompatible interactions. Interestingly, all but one of the identified genes are reported here for the first time to be involved in defense response to rice blast. In addition, hybridization of these clones with cDNAs synthesized from RNA samples from bacterial blight-infected leaves showed that few of them are induced or repressed in *Xa21*- or *Xa7*-resistant plants, suggesting a minimum overlap of defense responses mediated by different resistance genes to fungal and bacterial pathogens at an early stage of infection. Further characterization and functional analysis of these genes will enhance our understanding of the molecular mechanism of broad-spectrum resistance in rice.

Communicated by D.J. Mackill

G. Lu · C. Jantasuriyarat · B. Zhou · G.-L. Wang (✉)
Department of Plant Pathology,
The Ohio State University,
201 Kottman Hall, 2021 Coffey Road, Columbus, OH 43210, USA
e-mail: wang.620@osu.edu
Tel.: +1-614-2929280
Fax: +1-614-2924455

G. Lu
College of Plant Protection,
Fujian Agricultural and Forestry University,
350002 Fuzhou, P.R. China

## Introduction

Upon pathogen infection, the plant defense response activates complex biochemical and structural changes in plant cells. A large number of defense-related genes are up- or down-regulated during plant-pathogen interactions. The most common resistance reaction in plants is the hypersensitive response (HR), which localizes cell death at the infection site. The HR is correlated with a transient burst of active oxygen species, the activation of specific defense-related genes, an accumulation of antimicrobial compounds and the alteration of the plant cell wall (Dangl et al. 1996; Hammond-Kosack and Jones 1996; Yang et al. 1997). The local responses at the point of infection also trigger a subsequent non-specific resistance that occurs throughout the entire plant; this phenomenon is called systemic acquired resistance (SAR). The SAR is long-lasting and effective against a broad spectrum of pathogens. It is also correlated with the induction of defense-response genes in the uninfected leaves that protect the new tissues from secondary infections (Ryals et al. 1996).

The HR typically occurs in "gene-for-gene" plant-pathogen interactions (Flor 1971), in which resistance in a host cultivar is controlled by a parasite-specific resistance (*R*) gene matched by avirulent (*Avr*) genes in the pathogen. One possible explanation for the molecular basis of gene-for-gene interaction is the ligand and receptor model, where the *R* gene product acts as a receptor that recognizes a ligand or elicitor that is produced directly or indirectly by the pathogen's *Avr* gene (Baker et al. 1997). This interaction, then, is involved in complex signal transduction networks, and leads to alteration of the defense response gene expression in plant cells.

In the past decade, much effort has been made to study the signal transduction and defense gene expression in

plants (reviewed by McDowell and Dangl 2000; Glazebrook 1999, 2001). However, most of the research was conducted in dicotyledonous species such as *Arabidopsis*, tobacco, and tomato. Only a few signaling components, such as a small GTP-binding protein (OsRac1), MAP kinase (BWMK1), rab-specific GDP-dissociation inhibitors (OsGDIs), and one Myb family of transcription factors (JAmyb), were found to be associated with blast infection (He et al. 1999; Kawasaki et al. 1999; Kim et al. 1999; Lee et al. 2001). Recently, fungal elicitor-responsive genes from rice cell suspensions and genes induced by non-host pathogen *Pseudomonas syringae* pv. *syringae* have been found (Mauch et al. 1998; Kim et al. 2000; Schaffrath et al. 2000). Although these results provide us with new insight into the molecular mechanisms of the defense response in rice, a systematic and thorough search for defense genes is essential to better understand the concerted response in rice cells to pathogen invasion.

Using both cDNA differential screening and suppression subtractive hybridization (SSH) methods, Xiong et al. (2001) identified a total of 56 defense genes that are responsive to blast infection and to the treatment of benzothiazole and jasmonate acid.However, none of the genes were differentially expressed in resistant and susceptible plants. Using a similar approach, we attempted to isolate genes that are induced or suppressed in early defense response (from 12 to 24 h after inoculation) of the broad-spectrum resistance gene *Pi9(t)* (Liu et al. 2002). Forty-seven unique genes were identified from two subtracted libraries. Aside from one gene (*R1C4,* encoding ADP-ribosylation factor) that was previously identified in Xiong et al.'s study (2001), all identified genes are novel and have not yet been reported to be involved in response to rice blast. Importantly, some of these genes are indeed differentially expressed in resistant and susceptible plants. We further demonstrate that most of these genes are not involved in defense response to *Xanthomonas oryzae* pv. *Oryzae*, or *Xoo,* the causal agent of the bacterial blight disease. This defense gene collection provides us with an important genomic resource to profile gene expression during rice and rice blast interaction. Manipulation of these genes using transgenic approaches may lead to the engineering of novel and durable resistant rice cultivars.

## Materials and methods

### Plants and inoculation

Rice cultivars 75-1-127, carrying resistance gene *Pi9(t)*, and C101A51, carrying resistance gene *Pi2(t)* to *Magnaporthe grisea* and their corresponding susceptible recurrent cultivars, IR31917 and CO39, respectively, were used in this study (Mackill and Bonman 1992; Liu et al. 2002). Plants were grown in growth chambers under a daytime temperature of 25°C and a nighttime temperature of 20°C, 80% humidity, and 12 h light (500 µmol photons/$m^2$ per s). Three-week-old plants were inoculated with a blast spore suspension ($10^5$ spores/ml) of Philippine isolate PO6-6. The inoculated plants were placed in plastic bags for 24 h at 26°C, and subsequently transferred to the growth chamber under the same conditions mentioned above. Leaf tissue was harvested from the four cultivars at 0, 12, 24, 48, and 72 h after inoculation. After harvesting, all leaf samples were immediately frozen in liquid nitrogen.

### Suppression subtractive library construction and differential screen

SSH was performed using the PCR Select cDNA Subtraction Kit from Clontech (Palo Alto, Calif.). Two different SSH cDNA libraries were constructed according to the manufacturer's instructions. In the "forward subtractive cDNA library", *Pi9(t)*-resistant line 75-1-127 was used as a "tester" and the recurrent susceptible line IR31917 was used as a "driver". In the reverse subtractive cDNA library, IR31917 was used as a tester and 75-1-127 as a driver. Therefore, genes induced in the resistant reaction could be screened from the first library, and genes induced in the susceptible reaction could be identified from the second library. Total RNAs isolated 12 and 24 h post-inoculation were pooled equally, and converted into cDNA, using a cDNA synthesis kit from Invitrogen (Carlsbad, Calif.). The final PCR products were cloned into the pGEM-T easy vector (Promega, Madison, Wis.) and transformed, by electroporation, into DH10B *Escherichia coli* cells (Invitrogen).

Individual clones from the two subtractive libraries were randomly picked and stored in 384-well plates. For differential screening, the clones were transferred to Hybond-$N^+$ nylon membranes (Amersham, Piscataway, N.J.) and grown at 37°C for colony hybridization. Duplicate membranes were hybridized with two different probes: the forward-subtracted probe and the reverse-subtracted probe. In the case of the forward-subtracted probe, cDNA was synthesized from mRNA that was obtained from 75-1-127 as tester and IR31917 as driver plants. This probe was enriched for transcripts induced in resistant reactions. The reverse-subtracted probe was similarly prepared, except that the tester mRNA was derived from IR31917 and the driver mRNA from 75-1-127. This probe was enriched for transcripts that were induced in susceptible reactions. After the first screening, all positive clones, showing obviously different hybridization intensities, were picked to 96-well plates and re-screened using the same set of probes.

### Reverse Northern blot analysis

The cDNA inserts were PCR-amplified individually from positive clones with M13-F and M13-R primers. The PCR products were then loaded, in duplicate, on two 1% agarose gels. After electrophoresis, DNA was transferred to Hybond $N^+$ membranes. The DNA blot contained three control cDNAs: one resistant-induced cDNA (*F1A4*), one susceptible-induced cDNA (*R1A1*) and the *Act1* gene, a constitutively active rice actin gene (Wang et al. 1992). The *F1A4* and *R1A1* were isolated from the forward and reverse subtractive libraries, respectively (Table 1). The *Act1* gene was PCR amplified from a rice cDNA pool with the following primers: forward primer, 5′ CGTCTGCGATAATGGAACTGG 3', reverse primer 5′ CTGCTGGAATGTGCTGAGAGAT 3'. Duplicated blots were hybridized with $^{32}$P-labeled forward-subtracted and reverse-subtracted probes.

### RNA isolation and Northern blot analysis

Total RNA was extracted using the protocol described by Chomczynski and Sacchi (1987) with the following modifications: 1–3 g of leaf tissue was ground to a fine powder in liquid nitrogen and transferred to a tube with 15 ml of extraction buffer [4 M Guanidinium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sodium lauryl sarcosilate, 0.1 M $\beta$-mercaptoethanol]. Then 1.5 ml of 2 M NaOAc (pH 4.0), 15 ml of water-saturated phenol and 3 ml of chloroform:isoamylalcohol (24:1) were added, in sequence, to the tube. The contents were thoroughly mixed for approximately 15 s, incubated on ice for 15 min and then centrifuged at 15,000 *g* for 30 min at 4°C. After transferring the aqueous phase to a new

**Table 1** Identified SSH clones and their BLAST search results

| Clone | Accession number | Best homologue in the database[a] | Score | E value | Sequence identity (%) | Induction | |
|---|---|---|---|---|---|---|---|
| | | | | | | R[b] | S |
| **I. Cell rescue/defence/cell death and aging** | | | | | | | |
| F2A11 | CD645545 | s-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase [*Cucumis sativus*] (AB046595) | 95.5 | 1e-19 | 53 | ++ | + |
| F3H5 | CD645546 | s-adenosyl-L-methionine:carboxyl methyltransferase-like protein [*Arabidopsis thaliana*] (NM_120520) | 53.1 | 8e-07 | 25 | ++ | + |
| R1A1 | CD645547 | ALG-2 (apoptosis-linked gene) interacting protein 1 [*Rattus norvegicus*] (AF192757) | 30.4 | 6.4 | 40 | c | + |
| R1A3 | CD645548 | cytochrome P450 monooxygenase [*Zea mays*] (AJ004810) | 226 | 1e-58 | 72 | + | + |
| R1A8 | CD645549 | aluminum-induced protein [*Brassica napus*] (AB013447) | 50.1 | 9e-06 | 62 | + | ++ |
| R1B7 | CD645550 | cytochrome P450 monooxygenase [*Oryza sativa*] (AC068924) | 67.8 | 3e-11 | 96 | + | + |
| **II. Cellular communication/signal transduction** | | | | | | | |
| F1B2 | CD645551 | putative receptor-like protein kinase [*Arabidopsis thaliana*] (AC002521) | 177 | 4e-44 | 56 | + | + |
| F4D6 | CD645552 | ethylene receptor-like protein 2 [*Oryza sativa*] (AF420318) | 92.4 | 1e-18 | 100 | ++ | + |
| **III. Transcription** | | | | | | | |
| F3A3 | CD645553 | putative CREB-binding protein [*Arabidopsis thaliana*] (AB026645) | 53.9 | 6e-07 | 43 | – | – |
| R1A5 | CD645554 | homeobox gene [*Oryza sativa*] (AB007627) | 166 | 1e-40 | 80 | N | N |
| R1E11 | CD645555 | adaptin-related protein APT-10 [*Caenorhabditis elegans*] (NM_073165) | 34.7 | 0.64 | 26 | + | ++ |
| R3C9 | CD645556 | CDH1-D [ *Gallus gallus*] (AF421549) | 69.7 | 2e-22 | 54 | N | N |
| **IV. Cellular transport and transport mechanisms** | | | | | | | |
| R1C4 | CD645558 | ADP-ribosylation factor [*Oryza sativa*] (D17760) | 198 | 2e-50 | 100 | – | – |
| R1F3 | CD645559 | peptide/amino acid transporter [*Oryza sativa*] (AC079685) | 33.5 | 0.70 | 29 | N | N |
| R1H4 | CD645560 | Putative vesicle transport V-SNARE protein [*Oryza sativa*] (AP003381)) | 141 | 2e-33 | 100 | + | ++ |
| **V. Metabolism** | | | | | | | |
| F1A4 | CD645561 | alcohol dehydrogenase-like protein [*Oryza sativa*] (AP003825) | 69.3 | 1e-11 | 100 | + + | + |
| F1E1 | CD645562 | 3-methylcrotonyl-COA carboxylase non-biotinylated [*Arabidopsis thaliana*] (AF059510) | 184 | 2e-46 | 64 | + | + |
| F4F9 | CD645563 | alpha-galactosidase [*Oryza sativa*] (AB039671) | 334 | 2e-91 | 98 | + | + |
| R1G5 | CD645564 | polygalacturonase [*Zea mays*] (X66422) | 32.3 | 1.3 | 34 | N | N |
| R3A7 | CD645565 | choline acetyltransferase isoform 1 [*homo sapiens*] (NM_020986) | 31.2 | 6.7 | 32 | N | N |
| **VI. Energy** | | | | | | | |
| F1B3 | CD645566 | ribulose-5-phosphate-3-epimerase [*Oryza sativa*] (AC073556) | 192 | 2e-48 | 100 | N | N |
| F1D8 | CD645567 | phosphoenolpyruvate carboxykinase 4 [*Urochloa panicoides*] (AF136163) | 73.6 | 5e-13 | 87 | + | + |
| F2G8 | CD645568 | cytoplasmic aconitate hydratase [*Arabidopsis thaliana*] (AY136414) | 223 | 7e-58 | 87 | ++ | + |
| F2H9 | CD645569 | ferredoxin [*zea mays*] (AB035645) | 59.3 | 1e-08 | 81 | – – | – |
| **VII. Protein destination** | | | | | | | |
| F1E6 | CD645570 | Lon protease [*Dichanthelium lanuginasum*] (AF385580) | 225 | 9e-59 | 99 | ++ | + |
| F2H1 | CD645571 | cysteine protease inhibitor [*Oryza sativa*] (J03469) | 71.2 | 3e-12 | 100 | ++ | + |
| F4G5 | CD645572 | putative serine carboxypeptidase [*Arabidopsis thaliana*] (AC010556) | 47.8 | 4e-05 | 51 | N | N |
| R1A2 | CD645573 | ubiquitin-conjugating enzyme [*Nicotiana tabacum*] (AB026056) | 34.7 | 0.30 | 100 | – – | – |
| **VIII. Unknown function** | | | | | | | |
| F1B5 | CD645591 | hypothetical protein [*Ralstonia solanacearum*] (NP_520924) | 32.0 | 1.8 | 39 | + | + |
| F1D9 | CD645574 | high mobility group protein [*Oryza sativa*] (AF093632) | 40.8 | 0.004 | 100 | + | ++ |
| F1H1 | CD645575 | OSJNBa0038F22.7 [*Oryza sativa*] (AP002838) | 133 | 5e-31 | 77 | ++ | + |
| F2A10 | CD645576 | KIAA1345 protein [*Homo sapiens*] (AB037766) | 31.6 | 2.6 | 32 | + | + |
| F2C6 | CD645577 | putative nuclear protein [*Caenorhabditis elegans*] (NP_505552) | 31.2 | 2.7 | 47 | ++ | + |
| F2G12 | CD645578 | putative protein [*Oryza sativa*] (AJ307662) | 91.7 | 1e-38 | 100 | + | + |
| F3E6 | CD645579 | unknown protein [*Arabidopsis thaliana*] (AY096642) | 60.8 | 7e-09 | 40 | ++ | + |
| F3F9 | CD645580 | unkonwn protein [*Arabidopsis thaliana*] (AC010924) | 218 | 3e-56 | 60 | ++ | + |
| R1A9 | CD645581 | hypothetical protein [*Arabidopsis thaliana*] (AL132978) | 99.0 | 1e-20 | 45 | c | + |
| R1B2 | CD645582 | putative senescence-associated protein [*Pisum sativam*] (AB049723) | 167 | 2e-42 | 92 | N | N |
| R1B4 | CD645583 | hypothetical protein [*Chlamydophila pneumoniae*] (F81516) | 102 | 4e-21 | 56 | N | N |
| R1C7 | CD645584 | hypothetical protein [*Caenorhabditis elegans*] (AF078792) | 32.3 | 1.4 | 32 | + | + |
| R1G7 | CD645585 | hypothetical protein [*Oenothera elata*] (NC_002693) | 77.8 | 2e-22 | 100 | N | N |
| R2G8 | CD645586 | ptr-9 protein [*Caenorhabditis elegans*] (CAA79560) | 32.3 | 1.6 | 35 | – – | – |
| R3A6 | CD645587 | SOH1-like proteion [*Oryza sativa*] (AP004267) | 130 | 1e-29 | 55 | N | N |
| R3B12 | CD645588 | hypothetical protein [*Mus musculus*] (XP_196551) | 50.8 | 4e-06 | 83 | N | N |
| R3C10 | CD645589 | hypothetical protein [*Arabidopsis thaliana*] (H84522) | 218 | 3e-56 | 68 | – | – |

**Table 1** (continued)

| Clone | Accession number | Best homologue in the database[a] | Score | E value | Sequence identity (%) | Induction | |
|---|---|---|---|---|---|---|---|
| | | | | | | R[b] | S |
| R3E8 | CD645590 | hypothetical protein [*Arabidopsis thaliana*] "contain similarity to ARI, RING finger protein " (T02366) | 41.6 | 0.002 | 47 | + | ++ |
| **IX. No match** | | | | | | | |
| F2C8 | CD645557 | No homology found | | | | N | N |

The clones beginning with an "F" are from the forward subtractive cDNA library, with an "R" are from the reverse subtractive cDNA library. "+" means induced, "–" means suppressed, "N" means no change, "++" means highly induced, and "– –" means highly suppressed, "c" means no hybridization signal in Pi-9(t) plants

[a] Genes were grouped using the same functional classification used for Arabidopsis thaliana MIPS (http://www.mips.biochem.mpg.de)

[b] R is on 75–1-127 resistant plants, S is on IR31917 susceptible plants

centrifuge tube, an equal volume of isopropanol was added, mixed, and stored at −20°C for 1 h. Precipitated RNA was centrifuged at 13,000 *g* for 25 min, and the pellet was dissolved in 5 ml of extraction buffer. RNA was precipitated again in 5 ml of isopropanol at −20°C for 1 h, followed by centrifugation again at 13,000 *g* for 20 min. After washing the pellet with 75% EtOH, it was dissolved in DEPC-treated water. Ten micrograms of total RNA were loaded and separated on formaldehyde-agarose gels and transferred to Hybond $N^+$ membranes (Sambrook et al. 1989) before hybridizing with selected cDNA clones.

### cDNA probes prepared from bacterial blight-infected leaf tissues

Two-month-old greenhouse-grown IRBB21 (carrying the cloned bacterial blight resistance gene *Xa21*; Song et al. 1995) and IR24 (susceptible) plants were inoculated by clipping leaf tips with a pair of sterile scissors dipped in a culture of *Xoo* strain POX99 (Race 6) from the Philippines (0.5 O.D. at 590 nm). In the wounding control, leaves were clipped with scissors dipped in sterile $H_2O$. Leaf tissues at a 0.5 cm margin of scissor clip were collected 24 h after inoculation or wounding. Total RNA was isolated, using the RNeasy Plant Mini Kit from Qiagen (Valencia, Calif.), and used as template for cDNA synthesis, using the SMART cDNA construction kit from Clontech. For probes used for reverse Northern blot analysis, cDNAs were labeled with $^{32}P$ using the Megaprime DNA-labeling system (Amersham).

## Results

### Construction and differential screening of cDNA libraries

To isolate cDNAs corresponding to genes differentially regulated in resistant and susceptible interactions with *M. grisea*, both forward and reverse subtractive cDNA libraries were made. Ten 384-well microtiter plates were picked from each library and stored in a −80°C freezer. The average insert size was approximately 450 bp, ranging from 300 to 800 bp. Based on the first differential screening, 376 clones from the forward subtractive library were identified as showing stronger hybridization when the forward-subtracted probe was used for hybridization. By contrast, 282 clones from the reverse library were identified as showing a stronger hybridization signal when the reverse-subtracted probe was used. The above clones were re-picked to 96-well plates, and spotted onto nylon membranes for the second screening. Thirty clones with different expression patterns in the resistant and susceptible reactions were chosen for sequencing. Sequence analysis indicated that some clones were highly redundant in both libraries. For example, about 70% of clones in the forward library were those encoding an alcohol dehydrogenase (ADH)-like protein. Homologs of ALG-2 (apoptosis-linked gene) interacting protein 1 and cytochome P450 monooxygenase were two of the most redundant clones in the reverse library. To remove these clones from both libraries, their PCR amplified inserts were used as probes to hybridize the membranes containing positive clones isolated from the first screen, and only non-hybridizing clones were selected for sequencing. In this way, the majority of the redundant clones were effectively removed.

### Reverse Northern blot analysis and BLAST search of identified clones

To further confirm and characterize the cDNA clones selected from the colony hybridization, "reverse Northern" analysis was used (Xiao et al. 2001). Blots containing PCR products amplified from cDNA clones were hybridized separately with $^{32}P$-labeled forward and reverse subtracted cDNAs. Most of the selected clones from the forward library showed increased expression in the resistant plants, and most of the selected clones from the reverse library showed increased transcript expression in the susceptible plants. A representative example highlighting the difference between the hybridization patterns with the forward and reverse probes is shown in Fig. 1. The clones that showed different hybridization intensity between the two probes were selected for sequencing. Their expression patterns in resistant and susceptible reactions, based on Northern blot analysis, are listed in Table 1. These results were further confirmed by Northern blot analysis (see results below).

Sequences were submitted to the NCBI database, and the BLASTX program was used to search for known proteins homologous to the cDNA clones. A total of 47 unique genes were identified, and their BLAST search results are summarized in Table 1. Except for one sequence from the forward cDNA library, *F2C8*, all the sequences had hits in the database. However, some

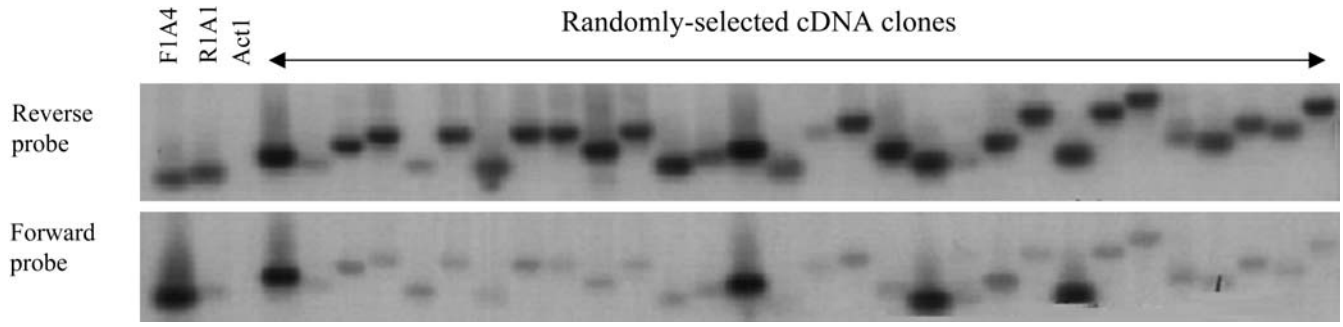


**Fig. 1** Reverse Northern blot analysis of cDNA clones selected from membrane hybridization. The cDNAs were amplified using M13 forward and reverse primers and loaded in duplicate on two 1% (w/v) agarose gels. Duplicated blots contained equal amounts of the PCR products and were hybridized with $^{32}$P-labeled forward- and reverse-subtracted probe, respectively. *F1A4* (induced in resistant plants), *R1A1* (induced in susceptible plants) and *Act1* (a constitutively expressed gene) were used as controls

clones, such as *R1A1, R1E11, R1F3, R1G5, R3A7, R1A2, F1B5, F2A10, F2C6, R1C7* and *R2G8,* showed a low matching score and large E-value. Most of these clones matched proteins in mammals. Whether they are the true homologs of the corresponding genes will require more experimental evidence. To verify whether any of these clones were derived from *M. grisea,* since tissue was harvested 12 and 24 h after inoculation, sequences of 47 clones were sent to the *M. grisea* Database at the Whitehead Institute (http://www-genome.wi.mit.edu/annotation/fungi/magnaporthe/). None of the sequences showed high homology with the fungal genome sequence. According to the putative function of top-hit homologs, 47 clones were further grouped into eight categories, using the same functional classification for *Arabidopsis thaliana* MIPS (http://www.mips.biochem.mpg.de).

Genes involved in "Cell rescue/defense/cell death and aging" were placed in category 1. In this group, two clones (*F2A11, and F3H5*), exhibiting stronger induction in the resistant reaction, were isolated from the forward library. The other four clones were isolated from the reverse library. Among them, only one (*R1A8*) was found to have differential expression with stronger induction in the susceptible reaction. It is interesting to note that two *s*-adenosyl-L-methionine:carboxyl methyl transferase (SACMT) genes, *F2A11*and *F3H5*, show 53% and 25% homology with SACMT of *Cucumis sativus* and *Arabidopsis thaliana,* respectively. Recently it was reported that the homologous gene in *Brassica carinata* is induced by $CuCl_2$ treatment, methyl jasmonate (MeJA), salicylic acid, and fungal pathogen infection (Zheng et al. 2001).

The second group contained genes involved in "Cellular communication/signal transduction". There are two receptor-like genes in this group that are homologous to *Arabidopsis* receptor-like protein kinase (*F1B2*) and rice ethylene receptor-like protein 2 (*F4D6*). None of these genes have been reported as being responsive to pathogen infection. Interestingly, although it was isolated from the reverse library, *F4D6* had a stronger induction in the resistant reaction.

There were four genes in the third category, "Transcription". Among them, *F3A3* was repressed while *R1E11* was strongly induced in the susceptible reaction. Neither *R1A5* nor *R3C9* showed induction after blast infection.

The fourth category was "Cellular transport and transport mechanisms". *R1F3* was not induced or repressed by blast infection. The homolog of ADP-ribosylation factor (*R1C4*) was repressed in both resistant and susceptible reactions, while the putative vesicle transport V-SNARE protein (*R1H4*) was strongly induced in the susceptible reaction.

The fifth group of genes consisted of those involved in "Metabolism". The alcohol dehydrogenase (ADH) gene is the only gene that was strongly induced in the resistant reaction. The ADH gene is induced during exposure to dehydration, cold, abscisic acid, and hypoxia in *Arabidopsis* (Dolferus et al. 1994; de Bruxelles et al. 1996).

Four genes were grouped in the sixth category, "Energy". The homolog of the *Arabidopsis* cytoplasmic aconitate hydratase gene was strongly induced in the resistant reaction. In contrast, the homolog of the maize ferredoxin gene was strongly repressed in the resistant reaction.

There were four genes in the category "Protein destination". These genes are homologs of *Dichanthelium lanuginosum* Lon protease, rice cysteine protease inhibitor, putative serine carboxypeptidase and ubiquitin-conjugating enzyme. The last group consisted of 18 genes with unknown function.

## Northern blot analysis of cDNAs during rice blast infection in *Pi9(t)*-resistant and -susceptible plants

To further confirm the results of the reverse Northern analysis and analyze the expression patterns during the rice and rice blast interactions at different time points, Northern blot analysis was conducted. RNA blots were made using total RNA extracted from *Pi9(t)*-carrying resistant 75-1-127 and susceptible IR31917 plants at six time points after blast inoculation (0, 4, 12, 24 48, 72 h). A total of 47 clones were used in the Northern blot analysis, 35 of them showed induction or repression after

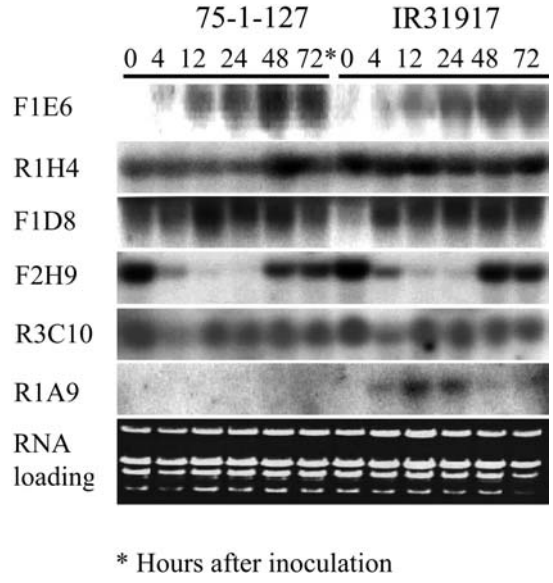


**Fig. 2** Northern blot analysis of some selected cDNA clones to show the typical expression pattern in *Pi9(t)*-resistant and -susceptible plants. Three-week-old plants of 75-1-127 (carrying the *Pi-9(t)* gene) and IR31917 (susceptible parent) were inoculated with blast isolate PO6-6 at a concentration of $10^5$ conidia/ml. RNA was isolated at indicated times, and approximately 10 $\mu$g RNA was separated on a denaturing agarose gel. The RNA blots were hybridized with the cDNA clones indicated at the *left* side. Ethidium bromide stained gel shows the equal loading of the RNA

blast inoculation. About 50% of the clones (23) showed different expression levels between resistant (75-1-127) and susceptible interactions (IR31917). According to their expression patterns, these genes can be further divided into six categories (Fig. 2). The first type includes those such as *F1E6* that are induced in both resistant and susceptible plants, but with stronger induction in resistant plants. The second type, such as *R1H*, includes those that are induced in both resistant and susceptible plants, but with stronger induction in susceptible plants. In the third type, such as *F1D8*, the genes are induced in both resistant and susceptible plants at similar levels. The genes suppressed in both resistant and susceptible plants, but with more suppression in resistant plants, comprise the fourth type, such as *F2H9*. In the fifth group, the genes are suppressed at the same level in both resistant and susceptible plants, such as *R3C10*. The last type, such as *R1A9*, is strongly induced in susceptible plants but has no detectable hybridization signal in resistant plants.

From the Northern blot analysis, we found that most of the genes are either early induced or repressed by the pathogen attack. For example, *F1E6* and *F1D8* were strongly induced as early as 4 h after inoculation (Fig. 2). On the other hand, the repression could also begin as early as 4 h after inoculation. For *F2H9,* the expression level was dramatically reduced at 4 h and almost completely repressed at 12–24 h, gradually increasing to a higher level at 48 and 72 h after inoculation. However, the expression pattern of *R3C10* was different in that the expression level was only reduced at 4 h after inoculation.

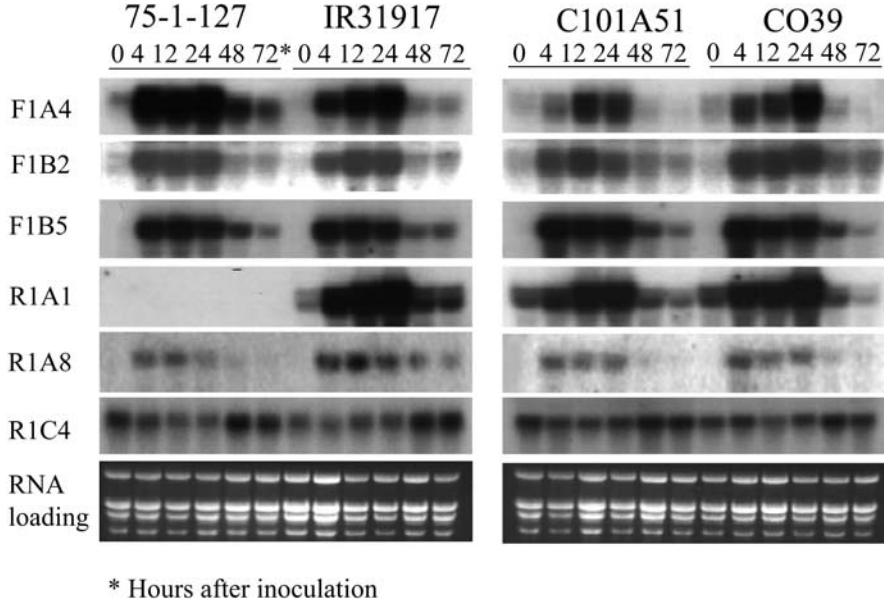


**Fig. 3** Northern blot analysis of some selected cDNA clones to compare expression patterns between *Pi9(t)*- and *Pi2(t)*-resistant and -susceptible plants. Three-week-old plants of *Pi9(t)*-resistant (75-1-127) and -susceptible (IR31917) plants, and *Pi2(t)*-resistant (C101A51) and -susceptible (CO39) plants were inoculated with blast isolate PO6-6 at a concentration of $10^5$ conidia/ml. RNA was isolated at indicated times, and approximately 10 $\mu$g RNA was separated on a denaturing agarose gel. The RNA blots were hybridized with the cDNA clones indicated at the *left* side. Ethidium bromide staining shows the equal loading of the RNA sample

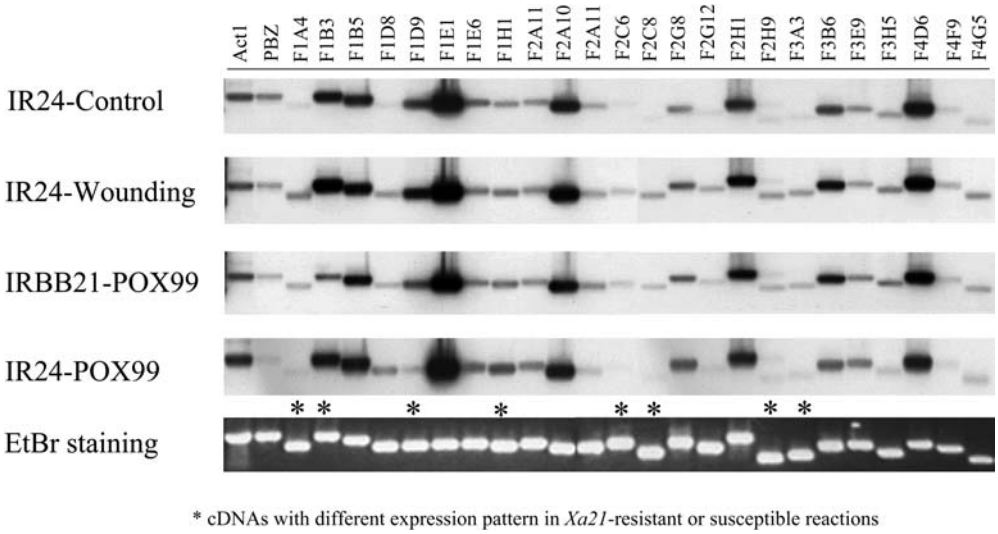


**Fig. 4** Reverse Northern blot analysis of clones from the forward library with *Xoo*-infected *Xa21*-resistant and -susceptible plants. PCR products (approximately 500 ng) were amplified from suppressive subtraction hybridization clones using M13F and M13R primers and loaded on 1.0% agarose gels. cDNAs synthesized from control, wounding, resistant and susceptible plants were labeled with $^{32}P$ and used as probes for hybridization with reverse Northern blots. Ethidium bromide staining shows the equal loading of the RNA samples

### Comparison of expression patterns in *Pi9(t)*- and *Pi2(t)*-resistant and -susceptible plants

Both *Pi9(t)* and *Pi2(t)* confer broad-spectrum resistance to rice blast, and are tightly linked on chromosome 6 (Liu et al. 2002). To test whether these *Pi9(t)*-associated defense genes are also involved in *Pi2(t)*-mediated resistance, several selected genes were used in Northern blot analysis with *Pi2(t)* resistant and susceptible plants. Expression patterns of six genes in both *Pi9(t)*- and *Pi2(t)*-resistant and -susceptible plants are shown in Fig. 3. With the exception of *F1A4* and *R1A1*, all tested genes had a similar expression pattern in four tested cultivars. *F1A4* encodes an ADH, and showed stronger expression in *Pi9(t)*-resistant plants (Fig. 3). However, its expression was a little bit higher in susceptible (CO39) than in *Pi2(t)*-resistant (C101A51) plants. *R1A1* encodes a protein matching an ALG-2 (apoptosis-linked gene) interacting-like protein from rat and did not have any expression in *Pi9(t)*-resistant plants, but was strongly induced in susceptible plants. Conversely, it was strongly induced in both *Pi2(t)*-resistant and -susceptible plants after inoculation. Its expression was induced as early as 4 h, and reduced to a normal level at 48 h (Fig. 3). To verify whether the *R1A1* gene is present in the *Pi9(t)* plants, a Southern blot analysis was conducted. The result indicated that the gene indeed exists in cultivar 75-1-127 and has 3–4 copies (data not shown). BLAST searches in the TIGR Rice Genome database confirmed the presence of the *R1A1* gene in the Nipponbare background. Further studies are warranted to investigate why *R1A1* is strongly suppressed even in uninoculated *Pi9(t)* plants.

### Expression of SSH genes in *Xa21*-resistant and -susceptible plants after bacterial blight infection

*Xa21* encodes a receptor-like protein (Song et al. 1995) and confers broad-spectrum resistance to diverse *Xoo* strains (Wang et al. 1996). To test whether the identified SSH genes are also responsive to *Xoo* infection, cDNA, synthesized from total RNA isolated from *Xa21*-resistant and -susceptible plants, was used as a probe in hybridization with the reverse Northern blots containing all 47 genes. cDNA synthesized from total RNA isolated from uninoculated and scissor-wounded plants was used as a control so that wounding-induced genes were removed from the comparison. Hybridization results showed that only 10 genes out of the identified 47 genes showed a small change in expression level 24 h after inoculation (Fig. 4, hybridization with reverse clones not shown). It is interesting to note that most of the affected genes had no visible change in the *Xa21*-resistant plants (third panel in Fig. 4) but were slightly repressed in susceptible plants (fourth panel in Fig. 4). The hybridization results with cDNA probes prepared from *Xa-7*-resistant and -susceptible plants had a similar expression pattern (data not shown). These results indicated that most of the genes isolated from the two SSH libraries are rice blast-specific, and are not involved in defense response mediated by bacterial blight resistance genes.

## Discussion

Over the last decade, the molecular mechanism for the defense response to rice blast has been studied using different approaches, such as homology cloning, differ-

ential display, cDNA library screening, and SSH methods (Zhu and Lamb 1991; He et al. 1999; Wang et al. 2001; Xiong et al. 2001). In 1991, Zhu and Lamb.(1991) isolated the first defense-related gene, encoding a basic rice chitinase, which was induced in suspension culture cells by a fungal cell wall elicitor. Since then, many defense-related genes that are responsive to blast infection have been identified. In this study, we used the SSH strategy to successfully isolate 47 defense-related genes from two subtractive libraries. Most of them showed differential expression patterns in the resistant and susceptible reactions. It is worth noting that out of these 47 genes, only *R1C4,* which encodes an ADP-ribosylation factor, has been previously identified to be involved in the defense response to rice blast (Xiong et al. 2001). The reason why we have identified so many new genes could be explained by the fact that different host cultivars, resistance genes and blast isolates were used in our study and other studies. The interaction between different pairs of the *R* gene and the corresponding *Avr* gene may lead to activation or suppression of a different set of defense genes at early infection stages. Alternatively, the sampling time of leaf tissue after inoculation was different among these studies.

To identify the early induced or suppressed genes from the rice-pathogen interaction, we combined RNA samples at 12 h and 24 h after inoculation to construct two SSH libraries. The Northern blot results confirmed that the transcript levels of most genes were changed before 24 h after inoculation. Some genes, such as *F1A4, F1B2, F1B5,* and *R1A8,* were strongly induced as early as 4 h post inoculation. In contrast, other studies, such as Xiong et al. (2001) and Kim and Lee (2001), used a mixture of RNA samples isolated from leaf tissue a few days after inoculation for cDNA and SSH library construction. Therefore, many of the pathogenesis-related genes that are induced in the late stages of blast infection were identified in their studies. Because the subtractive cDNA libraries were made with pooled RNA from 12 and 24 h infected leaves, some *M. grisea* genes may be intermingled in these clones. However, none of them showed high homology to the fungal genome sequence at the Whitehead Institute, probably due to little difference in pathogenic gene expression between resistant and susceptible plants at an early stage of infection (12 and 24 h after inoculation).

In this study, 47 unique genes were isolated and assigned to eight different groups according to the putative function of their homologous genes in the database. The clones in the defense and stress group were matched with several known pathogenesis-related proteins involved in plant defense. Both *F2A11* and *F3H5* encode a salicylic acid carboxyl methyltransferase-like protein. In *Brassica carinata*, the gene was induced by copper, pathogen infection, MeJA, and salicylic acid (Zheng et al. 2001). *R1A1* encodes a protein matching to *ALG-2* (apoptosis-linked gene) interacting protein 1 (*AIP1*) from rat, but with a low score and high E-value. ALG-2 is a $Ca^{2+}$-binding protein and is required for T-cell receptor-, Fas-, and glucocorticoid-induced cell death. It may mediate $Ca^{2+}$-regulated signals along the death pathway (Vito et al. 1996). *AIP1* interacts and cooperates with *ALG-2* in executing the calcium-dependent requirements along the cell death pathway (Vito et al. 1999). Overexpression of a deletion mutant of *AIP1* protects HeLa and COS cells from apoptosis, induced by serum starvation. In our experiment, *R1A1* is highly redundant in the reverse subtractive cDNA library, and strongly induced in the *Pi9(t)* susceptible plants, but it has a very low expression in *Pi9(t)*-resistant plant 75-1-127. Interestingly, this gene showed a similar expression pattern in the *Pi2(t)*-resistant and -susceptible plants. Because *R1A1* only has low homology to the rat *AIP1* gene, the function of this gene is unknown in rice. *R1A3* and *R1B7* encode the cytochome P450 monooxygenase-like protein (P450s). This type of gene has a high copy number in our reverse subtractive cDNA library. It is now clear that P450s form the largest class of plant enzymes. Some essential P450 functions are conserved among plant species, including hormone, sterol, and oxygenated fatty acid synthesis. Others, probably the majority, are involved in aspects of secondary metabolism that differ from plant to plant (Werck-Reichhart et al. 2000). A number of P450s have been shown to be induced by pathogen infection, and are involved in the synthesis of phytoalexins (Beyer et al. 2001). P450s also play an important role in the excretion of toxic substances into the vacuole (Beyer et al. 2001). Functional analysis of these genes, using overexpression or RNAi gene-silencing strategies, will elucidate their specific roles in defense response to rice blast.

In addition to the clones in the defense and stress groups, several other types of cDNA clones were also identified in our study and are potentially related to defense response. Most of these genes are signaling molecules that are involved in different biological processes. For example, two clones, which encode putative receptor-like protein kinase (*F1B2*) and ethylene receptor-like protein (*F4D6*), may be involved in cellular communication or signal transduction in plant defense. Four putative transcription factors (*F3A3, R1A5, R1E11* and *R3C9*) may interact with other defense genes, triggering downstream genes. *R1C4*, in the "Cellular transport and transport mechanisms" group, encodes ADP-ribosylation factor (ARF). The ARF proteins are members of the RAS family of small GTP-binding proteins that regulate various cellular functions in eukaryotes, such as vesicle formation and intracellular vesicle transport (McElver et al. 2000). This gene was also identified as a defense-related rice gene in a previous report (Xiong et al. 2001).

The ADH-like gene (*F1A4*) was highly redundant in our forward subtractive cDNA library. It was strongly induced by blast pathogen infection as early as 4 h post-inoculation (Fig. 3). ADH is a key enzyme in fermentation and anaerobic metabolism, where it contributes to ethanol production from acetaldehyde derived from pyruvate. Expression of the ADH gene in *Arabidopsis* is

induced during dehydration and at cold temperatures, as well as under conditions of hypoxia (Dolferus et al. 1994) and exogenous abscisic acid treatment (de Bruxelles et al. 1996). $Ca^{2+}$ and ethylene signaling are required for the activation of the Arabidopsis *ADH* gene (Chung and Ferl 1999; Peng et al. 2001). However, there is no previous report about this gene's involvement in disease resistance. *F1D8* encodes a homologous gene to phosphoenolpyruvate carboxykinase (PCK), an ATP-dependent enzyme, and the first enzyme in the gluconeogenic pathway in plants. The PCK gene was differentially screened from alfalfa and induced during early response to *Meloidogyne incognita* in roots of resistant and susceptible alfalfa cultivars (Potenza et al. 2001). *F2H9* encodes a protein that is homologous to ferredoxin:NADP(+) oxidoreductase, which participates in cellular defense against oxidative damage (Krapp et al. 1997). During hypersensitive reaction to pathogens, it is known that plants rapidly produce and accumulate reactive oxygen species, such as $O_2^-$ and $H_2O_2$ in the early stages after pathogen attack (Dangl and Jones 2001). Whether this gene plays any role in protecting rice cells from oxidative burst damage requires further investigation. *F2H1* has 100% identity with the rice cysteine protease inhibitor gene. In soybean, this gene is induced by wounding and MeJA treatment, and is presumed to have a role in plant defense (Botella et al. 1996). It is interesting to note that a part of the *R1A2* sequence shows 100% identity with the ubiquitin-conjugating enzyme of tobacco. Recently, growing evidence showed that the ubiquitin-proteasome pathways plays an important role in plant defense response (Austin et al. 2002; Kim and Delaney 2002; Peart et al. 2002; Xu et al. 2002). Further characterization of this gene may shed light on the relationship between the ubiquitination pathway and the defense response in rice.

Both bacterial blight and rice blast are important rice pathogens. It is still unknown whether infection from both pathogens will trigger a similar set of defense response genes or not. In this study, we isolated RNA samples from both *Xa21* and *Xa7* infected plants and used the synthesized cDNA for reverse Northern analysis with 47 blast-responsive genes. It is interesting to note that only few of the SSH genes showed changes in *Xa2-1* or *Xa7*-resistant and -susceptible plants after inoculation, suggesting a minimum overlapping of defense pathways mediated by different resistance genes to fungal and bacterial pathogens at an early stage of infection.

In summary, the SSH method has allowed us to generate two cDNA libraries, highly enriched for defense-related cDNAs from rice seedlings at a early stage of both resistant and susceptible interactions with rice blast. Twenty-three of 47 cDNAs showed differential expression between the compatible and incompatible interactions. Out of 47 genes identified, 46 are first reported to be induced or repressed in rice blast infection, demonstrating that the SSH method is an effective way to isolate genes that are differentially expressed in defense response to pathogen infection. Further characterization and functional analysis of these clones will enhance our understanding of the defense response mechanism in rice plants.

**Acknowledgements** We thank Drs. Bing Yang and Frank White for providing RNA samples isolated from *Xoo*-infected *Xa7* plants. Special thanks go to E. Mazur for critical reading of the manuscript. This project is, in part, supported by a grant from the Ohio State University's Ohio Agricultural Research and Development Center (OARDC-Industry Matching), the Young Professor Award from DuPont Inc. to GLW, and Natural Science Foundation of Fujian province to GL (no. 2003F008).

## References

Austin MJ, Muskett P, Kahn K, Feys BJ, Jones JD, Parker JE (2002) Regulatory role of *SGT1* in early R gene-mediated plant defenses. Science 295:2077–2080

Baker B, Zambryski P, Stakawicz B, Dinesh-Kumar SP (1997) Signaling in plant-microbe interactions. Science 276:726–733

Beyer K, Binder A, Boller T, and Collinge M (2001) Identification of potato genes induced during colonization by *Phytophthora infestans.* Mol Plant Pathol 2:125–134

Botella MA, Xu Y, Prabha TN, Zhao Y, Narasimhan ML, Wilson KA, Nielsen SS, Bressan RA, Hasegawa PM (1996) Differential expression of soybean cysteine proteinase inhibitor genes during development and in response to wounding and methyl jasmonate. Plant Physiol 112:1201–1210

Bruxelles GL de, Peacock WJ, Dennis ES, Dolferus R (1996) Abscisic acid induces the alcohol dehydrogenase gene in *Arabidopsis*. Plant Physiol 111:381–391

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-choloform extraction. Anal Biochem 162:156–159

Chung HJ, Ferl RJ (1999) *Arabidopsis* alcohol dehydrogenase expression in both shoots and roots is conditioned by root growth environment. Plant Physiol 121:429–436

Dangl JL, Jones JD (2001) Plant pathogens and integrated defence responses to infection. Nature 14:826–833

Dangl JL, Dietrich RA, Richberg MH (1996) Death don't have no mercy: Cell death programs in plant-microbe interactions. Plant Cell 8:1793–1807

Dolferus R, Jacobs M, Peacock WJ, Dennis ES (1994) Differential interactions of promoter elements in stress responses of the *Arabidopsis Adh* gene. Plant Physiol 105:1075–1087

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9:275–296

Glazebrook J (1999) Genes controlling expression of defense responses in *Arabidopsis*. Curr Opin Plant Biol 2:280–286

Glazebrook J (2001) Genes controlling expression of defense responses in *Arabidopsis*—2001 status. Curr Opin Plant Biol 4:301–308

Hammond-Kosack KE, Jones JDG (1996) Resistance gene-dependent plant defense responses. Plant Cell 8:1773–1791

He C, Fong SH, Yang D, Wang GL (1999) BWMK1, a novel MAP kinase induced by fungal infection and mechanical wounding in rice. Mol Plant Microbe Interact 12:1064–1073

Kawasaki T, Henmi K, Onstenk J, Hatakeyama S, Iwano M, Satoh H, Shimamoto K (1999) The small GTP-binding protein Rac is a regulator of cell death in plants. Proc Natl Acad Sci USA 96:10922–10926

Kim HS, Delaney TP (2002) *Arabidopsis* SON1 is an F-box protein that regulates a novel induced defense response independent of both salicylic acid and systemic acquired resistance. Plant Cell 14:1469–1482

Kim IP, Lee YH (2001) Analysis of genes expressed during rice-*Magnaporthe grisea* interactions. Mol Plant Microbe Interact 14:1340–1346

Kim CY, Lee SH, Park HC, Bae CG, Cheong YH, Choi YJ, Han C, Lee SY, Lim CO, Cho MJ (2000) Identification of rice blast

fungal elicitor-responsive genes by differential display analysis. Mol Plant Microbe Interact 13:470–474

Kim WY, Kim CY, Cheong NE, Choi YO, Lee KO, Lee SH, Park JB, Nakano A, Bahk JD, Cho MJ, Lee SY (1999) Characterization of two fungal-elicitor-induced rice cDNAs encoding functional homologues of the rab-specific GDP-dissociation inhibitor. Planta 210:143–149

Krapp AR, Tognetti VB, Carrillo N, Acevedo A (1997) The role of ferredoxin-NADP+ reductase in the concerted cell defense against oxidative damage—studies using *Escherichia coli* mutants and cloned plant genes. Eur J Biochem 249:556–563

Lee MW, Qi M, Yang Y (2001) A novel jasmonic acid-inducible rice *myb* gene associates with fungal infection and host cell death. Mol Plant Microbe Interact 14:527–535

Liu G, Lu G, Zeng L, Wang GL (2002) The two broad-spectrum blast resistance genes, *Pi9(t)* and *Pi2(t)*, are physically linked on rice chromosome 6. Mol Gen Genom 267:472–480

Mackill DJ, Bonman JM (1992) Inheritance of blast resistance in near-isogenic lines of rice. Phytopathology 82:746–749

Mauch F, Reimmann C, Freydl E, Schaffrath U, Dudler R (1998) Characterization of the rice pathogen-related protein *Rir1a* and regulation of the corresponding gene. Plant Mol Biol 38:577–586

McDowell JM, Dangl JL (2000) Signal transduction in the plant immune response. Trends Biochem Sci 25:79–82

McElver J, Patton D, Rumbaugh M, Liu C, Yang LJ, Meinke D (2000) The *TITAN5* gene of *Arabidopsis* encodes a protein related to the ADP ribosylation factor family of GTP binding proteins. Plant Cell 12:1379–1392

Peart JR, Lu R, Sadanandom A, Malcuit I, Moffett P, Brice DC, Schauser L, Jaggard DA, Xiao S, Coleman MJ, Dow M, Jones JD, Shirasu K, Baulcombe DC (2002) Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. Proc Natl Acad Sci USA 99:10865–10869

Peng HP, Chan CS, Shih MC, Yang SF (2001) Signaling events in the hypoxic induction of alcohol dehydrogenase gene in *Arabidopsis*. Plant Physiol 126:742–749

Potenza C, Thomas SH, Sengupta-Gopalan C (2001) Genes induced during early response to *Meloidogyne incognita* in roots of resistant and susceptible alfalfa cultivars. Plant Sci 161:289–299

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steriner H-Y, Hunt MD (1996) Systemic acquired resistance. Plant Cell 8:1809–1819

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Schaffrath U, Mauch F, Freydl E, Schweizer P, Dudler R (2000) Constitutive expression of the defense-related *Rir1b* gene in transgenic rice plants confers enhanced resistance to the rice blast fungus *Magnaporthe grisea*. Plant Mol Biol 43:59–66

Song WY, Wang GL, Chen L, Zhai W, Kim HK, Holsten T, Zhu L, Ronald PC (1995) The rice disease resistance gene, *Xa21*, encodes a receptor-like protein kinase. Science 270:1804–1806

Vito P, Lacana E, D'Adamio L (1996) Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3. Science 271:521–525

Vito P, Pellegrini L, Guiet C, D'Adamio L (1999) Cloning of AIP1, a novel protein that associates with the apoptosis-linked gene ALG-2 in a Ca2+-dependent reaction. J Biol Chem 274:1533–1540

Wang GL, Song W, Ruan D, Sideris S, Ronald PC (1996) The cloned gene, *Xa21*, confers resistance to multiple *Xanthomonas oryzae pv. oryzae* isolates in transgenic plants. Mol Plant Microbe Interact 9:850–855

Wang Y, Zhang W, Cao J, McElroy D, Wu R (1992) Characterization of cis-acting elements regulating transcription from the promoter of a constitutively active rice actin gene. Mol Cell Biol 12:3399–406

Wang Z, Taramino G, Yang D, Liu G, Tingey SV, Miao GH, Wang GL (2001) Rice ESTs with disease resistance gene- or defense response gene-like sequences mapped to regions containing major resistance genes or QTLs. Mol Genet Genomics 265:302–310

Werck-Reichhart D, Hehn A, Didierjean L (2000) Cytochomes P450 for engineering herbicide tolerance. Trends Plant Sci 5:116–123

Xiao F, Tang X, Zhou JM (2001) Expression of 35S::Pto globally activates defense-related genes in tomato plants. Plant Physiol 126:1637–1645

Xiong L, Lee MW, Qi M, Yang Y (2001) Identification of defense-related rice genes by suppression subtractive hybridization and differential screening. Mol Plant Microbe Interact 14:685–692

Xu L, Liu F, Lechner E, Genschik P, Crosby WL, Ma H, Peng W, Huang D, Xie D (2002) The SCF(COI1) ubiquitin-ligase complexes are required for jasmonate response in *Arabidopsis*. Plant Cell 14:1919–1935

Yang Y, Shah J, Klessig DF (1997) Signal perception and transduction in plant defense responses. Genes Dev 11:1621–1639

Zheng Z, Uchacz TM, Taylor JL (2001) Isolation and characterization of novel defence-related genes induced by copper, salicylic acid, methyl jasmonate, abscisic acid and pathogen infection in *Brassica carinata*. Mol Plant Pathol 2:159–169

Zhu Q, Lamb CJ (1991) Isolation and characterization of a rice gene encoding a basic chitinase. Mol Gen Genet 226:289–296